The Early Natural History of Nephropathy in Type 1 Diabetes

II. Early Renal Structural Changes in Type 1 Diabetes

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Renal structural abnormalities are known to precede the development of proteinuria, hypertension, and reduced renal function in patients with type 1 diabetes. The determinants of these early structural abnormalities are, however, largely unknown. The International Diabetic Nephropathy Study (IDNS) has recruited 243 children and adults (aged 10–40 years) in Montreal, Minneapolis, and Paris to identify and quantify these determinants. All study subjects were normotensive and had normal-to-high glomerular filtration rates (GFRs) and urinary albumin excretion rates (AERs) <100 µg/min at study entry. Only 8 of 243 had an AER ≥20 µg/min (microalbuminuria). Two renal biopsies are obtained at a 5-year intervals, with baseline and follow-up measures of renal function, blood pressure (BP), HbA$_{1c}$, plasma lipids, and AER. Herein, we examine the baseline renal biopsy morphometric findings in these subjects and in 87 kidney donor control subjects and explore the associations between findings and clinical and demographic variables. The principal morphometric abnormalities were increased glomerular basement membrane (GBM) width and fractional volume of mesangium [Vv(Mes/glom)] and mesangial matrix [Vv(MM/glom)]. The frequency of these abnormalities increased with increasing duration of diabetes but was observed as early as 2–8 years after onset. Diastolic BP (DBP), but not HbA$_{1c}$, was directly associated with these abnormalities. Elevated GFR was associated with only a small increase in peripheral glomerular capillary basement membrane filtration surface density. Center differences were detected in renal structural, renal functional, and BP parameters, especially between the Paris and North American centers. GBM width, Vv(Mes/glom), and Vv(MM/glom) are significantly increased even within a few years of onset of type 1 diabetes. These changes are detectable in normoalbuminuric patients and are related to duration, BP, and study site. Changes in these and other morphometric measures over 5-year follow-up should help clarify the roles of glycemia and other determinants of the rates of development of diabetic nephropathy lesions, as well as their relationships to early changes in BP, albumin excretion, and renal function. *Diabetes* 51:1580–1587, 2002

Diabetes, the leading cause of end-stage renal disease (ESRD) in the U.S., accounts for 44% of all new patients entering ESRD programs (1). Of the patients with type 1 diabetes, ~25% develop diabetic nephropathy (DN) and progress to ESRD (2,3). Because age at onset of type 1 diabetes is typically much younger than that of type 2 diabetes, ESRD most often develops at an earlier age. Thus, the burden to patients and to society is particularly severe, occurring during the period of productive work and family responsibilities (2).

A central concept in understanding the pathogenesis of DN is that it results from a series of specific progressive renal pathological changes that have their onset early in the course of diabetes (4). These changes develop during a long silent period, before features of clinical renal disease—including proteinuria, hypertension, and declining glomerular filtration rate (GFR)—are evident (2,4,5). Central to the nephropathological changes is the accumulation of extracellular matrix (6), leading to expansion of glomerular mesangial regions at the expense of filtration surface area (7,8), and thickening of the glomerular (2,4) and tubular basement membranes (9). Important changes in arterioles (2,10) and in the renal interstitium (11) also occur. Although all of these structural parameters will be addressed in these studies, the key end point measurements are those related to mesangial expansion, because this is the pathological process most closely related to the development of clinical renal disease and to loss of filtering function in type 1 diabetic patients (2,4,5,7,8).

At the advanced stage of DN, when renal insufficiency is present, the specific factors responsible for the genesis of early diabetic renal lesions may be subsumed by mechanisms common to progression in many different forms of advanced renal disease (12). Thus, to understand the genesis of the renal lesions of DN, they must be examined in their preclinical phases, before the process becomes

blurred by mechanisms that are not specific to diabetes. Previous studies of early renal pathological changes in type 1 diabetes have included relatively small numbers of patients, have usually been based in a single country, and have rarely included children. The International Diabetic Nephropathy Study (IDNS) involves a large number of diabetic subjects from three widely separated university medical centers (Montreal, Minneapolis, and Paris), and includes both children and adults who are free of overt DN at baseline. The study uses a prospective cohort design with measurement of the rate of development of renal lesions over time in order to assess the contributions of many potential pathophysiological determinants. Here, we present the baseline renal biopsy results from this study and the cross-sectional analysis of those determinants.

RESEARCH DESIGN AND METHODS

In an accompanying article (13) in this issue of *Diabetes*, we provide a detailed description of the study design; inclusion and exclusion criteria and recruitment methods; demographic characteristics of the study cohort; renal biopsy procedure and its complications; methods used to measure GFR, renal plasma flow (RPF), filtration fraction (FF), blood pressure (BP), HbA$_{1c}$, and albumin excretion rate (AER); and the results of these measurements. The demographic and clinical characteristics of the patients in this study are summarized in Table 1. Briefly, the cohort included 243 type 1 diabetic subjects whose ages ranged from 10 to 40 years and whose duration of diabetes ranged from 2 to 20 years. All were normotensive at baseline, had normal or elevated GFR, and had AER <100 μg/min.

Normal control subjects. Tissue was obtained by renal biopsy from 87 normal donor kidney control subjects at the time of renal transplantation. The control subjects, who were aged 24 ± 8.6 years (range 8–47), were older on average than the diabetic cohort, who were aged 17 ± 6.0 years (10–38) ($P = 0.0001$).

Renal morphometric measures. Of the 243 subjects fulfilling the entry criteria including adequate tissue for electron microscopy (EM), adequate tissue was also obtained for cortical interstitial volume and glomerular volume (GlomVol) by light microscopy in 211 and 197 subjects, respectively.

Tissue for EM morphometry at all three centers was fixed in 2.5% glutaraldehyde in Millonig's buffer and processed identically for EM as previously described (5). All EM sectioning, staining, and image acquisition were performed at the University of Minnesota, and all measurements were carried out by a single observer without knowledge of the tissue source. Sections (1-μm) were stained with toluidine blue. If an entire glomerular profile was present in the first technically good section, it was used for EM. If not, ~80 1-μm sections were cut and discarded before the next section was saved and stained. If a new glomerulus was present, it was sectioned for EM, but if no new glomerulus was present, this sectioning process was repeated. This sampling protocol eliminated bias that could result from using sections from a particular region of the glomerulus.

The entire glomerular profile was photographed using a 100CX electron microscope (JEOL, Tokyo) for measurement of the volume fraction of mesangium per glomerulus [Vv(Mes/glom)] and the surface density of peripheral glomerular basement membrane [Sv(PGBM/glom)] (5). These micrographs were printed at $3,900\times$ and assembled into a montage of the entire glomerular cross-section. All montages were blindly screened by one of the investigators (M.M.) to eliminate measurement of sclerotic, compressed, or torn glomeruli or glomeruli with other serious artifacts. Micrographs for measuring GBM width and mesangial components were obtained as previously described and printed at $11,000\times$ (5). A calibration grid was photographed at both low and high magnifications and printed with each roll of film to determine the precise magnification of all micrographs. If present, three glomeruli were measured from each biopsy (three biopsies had only two glomeruli). After measuring three glomeruli, if there were <150 coarse points (see below) hitting these glomeruli, additional glomeruli were added, if present, until at least 150 coarse points were counted. An average of 3.7 (range 2–6) glomeruli per biopsy were measured. Tissue for light microscopy was fixed in Zenker's (Minneapolis center) or Brazil's (Montreal and Paris centers) solution and embedded in paraffin. Each biopsy was cut completely into 5-μm sections, and all sections were saved, sequentially numbered, and stained with periodic acid Schiff (PAS).

Glomerular basement membrane (GBM) width was measured in nm using the orthogonal intercept method of Jensen et al. (14). An average of 169 (range 70–355) measurements per biopsy were evaluated.

Vv(Mes/glom) was measured using point counting on the low-magnification montages (5). A double-lattice grid consisting of coarse points (6 mm apart) and fine points (3 mm apart) was randomly placed over the montage. There were four fine points for each coarse point. Points were summed over all glomeruli from a biopsy, and the volume density was calculated using the equation:

$$\text{Vv(Mes/glom)} = \text{FP}_\text{M}/(\text{CP}_\text{G} \times 4)$$

where FP_M is the sum of fine points hitting the mesangium and CP_G is the sum of coarse points hitting the glomerulus. An average of 196 (range 99–357) coarse points and 172 (75–400) fine points per biopsy were counted.

Volume density of mesangial components per glomerulus. The mesangial region was divided into mesangial cell (MC), mesangial matrix (MM), and mesangial GBM (MGBM) and estimated by point counting using a grid with points 3 mm apart randomly placed over the high-magnification micrographs. The volume density for each of these three components was calculated using the following equations:

$$V_v(\text{MC/mes}) = P_\text{MC}/(P_\text{MC} + P_\text{MM} + P_\text{MGBM})$$

$$V_v(\text{MM/mes}) = P_\text{MM}/(P_\text{MC} + P_\text{MM} + P_\text{MGBM})$$

$$V_v(\text{MGBM/mes}) = P_\text{MGBM}/(P_\text{MC} + P_\text{MM} + P_\text{MGBM})$$

where P_MC, P_MM, and P_MGBM are the number points hitting cell, matrix, and MGBM, respectively. The volume density per mesangium was multiplied by Vv(Mes/glom) to obtain the volume density per glomerulus for each of the mesangial components. Only MC and MM data are presented.

Sv(PGBM/glom). Sv(PGBM/glom) was estimated on the montages using intercept counting (4). A grid of lines was randomly placed over each montage, and the number of intercepts of these lines with the epithelial side of the peripheral basement membrane was noted. Surface density was calculated as:

$$\text{Sv(PGBM/glom)} = 2 \times \text{I}/(\text{CP}_\text{G} \times 60{,}000/\text{Mag})$$

where I is the number of intercepts of a grid line with the peripheral GBM, CP_G is the number of coarse points hitting a glomerulus, 60,000 is the length in micrometers of the grid line represented by each coarse point, and Mag is the magnification of the montage. An average of 218 (range 85–387) intercepts per biopsy was counted.

Total surface per glomerulus of the peripheral GBM (TFS) was calculated as:

$$\text{TFS} = \text{Sv(PGBM/glom)} \times \text{GlomVol in } \mu\text{m}^2 \times 10^3$$

GlomVol. GlomVol was measured by the Cavalieri method by light microscopy by a single observer at the University of Minnesota, as detailed elsewhere (15), without knowledge as to the tissue source. Every fourth section was viewed; as new glomeruli appeared, they were numbered, and a grid of points was randomly superimposed over each new profile. The number of points hitting each profile was noted. GlomVol (in cubic micrometers) was calculated using the equation:

$$\text{GlomVol} = 20 \times \Sigma P_g \times (5{,}000/150)^2 \text{ in } \mu\text{m}^3 \times 10^6$$

where 20 is the interval in micrometers between each fourth section, ΣP_g is the sum of grid points hitting all profiles from a glomerulus, 5,000 is the

TABLE 1
Demographic and clinic characteristics of the study participants

Characteristic	
F/M (%)	51/49
Caucasian (%)	96
Age at entry (years)	16.8 ± 6.0
Age at onset (years)	8.8 ± 4.8
Duration (years)	8.0 ± 4.2
HbA$_{1c}$ (%)*	8.7 ± 1.5
AER (μg/min)	7.6 ± 12.5
GFR (ml · min^{-1} · 1.73 m^{-2})†	142 ± 28
SBP (mmHg)‡	114 ± 11
DBP (mmHg)‡	63 ± 9

Values are means $\pm$ SD. *$n = 241$; †$n = 237$; ‡$n = 242$.

distance in micrometers between grid points, and 150 is the magnification. The mean GlomVol was calculated from all the glomeruli measured from a biopsy. For a biopsy to be included, at least three glomeruli had to be measured. A total of 204 biopsies had at least three measurable glomeruli. An average of 13.1 (range 3–25) glomeruli provided the estimate of GlomVol. For quality control, GlomVol was measured in 26 biopsies fixed in both Brazil and Zenker's solutions. The correlation of GlomVol values in these two solutions was $r = 0.78$ ($P < 0.001$). Values for GlomVol were consistently and systematically greater in the Brazil compared with the Zenker's fixative. Bland-Altman plots indicated no trend or deviation. To adjust for this difference, a correction factor derived from the regression analysis was therefore applied to all the GlomVol calculations for tissue fixed in the Zenker's solution as follows: GlomVol (Zenker's) $\times$ (1.578 − 0.486) and only corrected data are presented. Calculations using GlomVol were weighted for the number of glomeruli measured.

Interstitial fractional volume. Interstitium is defined as that portion of the cortex not composed of glomeruli, tubules, or arteries or veins with a diameter at least that of a tubule. Interstitial fractional volume [Vv(Int/cortex)] was determined in 209 biopsies by a single blinded observer at the University of Minnesota by point counting a single light microscopy section using a projection microscope and a double-lattice grid consisting of coarse points (25 mm apart) and fine points (12.5 mm apart) (11). The microscope stage was systematically advanced until the entire tissue section had been measured. There were four fine points for each coarse point. Vv(Int/cortex) was calculated using the following formula:

$$Vv(\text{Int/cortex}) = FP_I/(CP_C \times 4)$$

where FP_I is the number of fine points hitting interstitium and CP_C is the number of coarse points hitting cortex. An average of 370 coarse points (range 74–1,154) and 140 fine points (23–578) were counted.

The index of arteriolar hyalinosis (IAH) was obtained by two observers [M.M. and Marie-Claire Gubler (M.-C.G.)] who, reading slides together, agreed upon the subjective estimate of the fraction of each arteriolar wall replaced by hyaline in one complete light microscopic section. Since higher scores for IAH are associated with more global glomerular sclerosis (GS) (10), greater weighting was given to arterioles with higher scores according to the formula:

Numerator: 1 $\times$ number of arterioles with a score $\leq$0.25
  + 2 $\times$ number of arterioles with a score 0.26–0.50
  + 3 $\times$ number of arterioles with a score 0.51–0.75
  + 4 $\times$ number of arterioles with a score $\geq$0.76
Denominator: Total number of arterioles counted

We examined 17 (range 2–102) vessels per biopsy.

The percent global GS was determined by two observers (M.M. and M.-C.G.) on the light microscopic sections used for IAH. A mean of 12 (range 1–67) glomeruli were examined per biopsy. Normal values (2.1 ± 3.9%) were as previously reported (10).

Kidney length. The length of each kidney (KL) was obtained by ultrasound at the time of the renal biopsy, and the mean of the left and right KL's was calculated. Published values for children adjusted for body surface area (BSA) were used to define normal KL (16) because KL is highly correlated with somatometric parameters in normal children (17). Normal adult KL values were derived from published data (18,19), and correlations with renal functional or structural data were adjusted for BSA up to values of 1.73 m^2 for both children and adults.

Statistical methods. Statistical comparisons of diabetic patients versus normal control subjects were based on two-tailed t tests. Multiple linear regression analysis for analysis of determinants of morphometric measures within the patient group was used. Regression models were tested with either SBP or DBP. Results were similar but were more consistent for DBP; therefore, results reported below are based on regression models containing DBP.

There was no relationship of any morphometric measures to age in the normal control group, except for GBM, where a nonlinear relationship with age was observed. Because age is highly collinear with duration of diabetes, multiple regression models contained a term for disease duration but not age. The mean values during the first year of the study were used for AER, HbA$_{1c}$, and BP in all analyses. Only 8 of the patients were microalbuminuric during the first year of the study, but 21 patients had insufficient data for classification. Results of the analyses were similar, whether these eight patients were included or omitted. Therefore, all patients were included in the analyses, except when the microalbuminuric and normoalbuminuric patients were compared. All statistical analyses were carried out using SAS-PC.

RESULTS

Morphometric findings. Values for GBM width, Vv(Mes/glom), fractional volume of MM [Vv(MM/glom)], and SvPGBM were greater in the diabetic subjects than in the normal control subjects (Table 2). After adjusting for age or age and BSA, the difference for Sv(PGBM) was no longer significant. Values for Vv(MC/glom), GlomVol, and TFS did not differ between these two groups. Vv(Int/cortex) was lower in the diabetic group than in the normal control subjects. All comparisons with control subjects remained the same when only normoalbuminuric patients were considered.

Table 3 shows the frequency (%) of diabetic subjects with abnormally high (>95th percentile) or low (<5th percentile) morphometric values in relation to duration of diabetes. Of note is the occurrence of abnormal values within 2–8 years of diabetes onset. SvPGBM was increased early in the course of type 1 diabetes but fell with longer duration of diabetes; by 14–20 years, 20% of diabetic subjects had values below normal. The most frequent abnormalities seen at 14–20 years were thickened GBM and increased Vv(MM/glom) (68% were abnormal for both).

Predictors of morphometric findings. Duration of diabetes and DBP were related to all glomerular morphometric measures except Vv(MC/glom) (Table 4). Female sex was related to Vv(MM/glom) and Vv(Int/cortex). Vv(Int/cortex) and Vv(MC/glom) were also positively associated with HbA$_{1c}$.

Few diabetic patients had arteriolar disease. The IAH score was 1 (i.e., no abnormalities) in 178 and >1 in 44 patients, with 10 patients having scores $\geq$1.1 and only 6 having scores $\geq$1.2. The group with arteriolar abnormalities (Table 5) had longer diabetes duration and more severe glomerular lesions than the group with normal arterioles. Similarly, few patients had increased percent GS (207 with $\leq$5% GS, 17 with $\geq$5% GS). Subjects with $\geq$5% GS had longer diabetes duration, greater GBM thickening, lower Sv(PGBM/glom), and greater Vv(Int/cortex) (Table 6). There were no significant associations between percent GS and IAH.

Study center differences. Differences in morphometric measures were observed among the study sites, particularly between Paris and the two North American centers (Tables 4 and 7). Vv(Mes/glom) and Vv(MC/glom) were

TABLE 2
Comparison of baseline morphometric findings in diabetic subjects and control subjects

Variable	Type 1 diabetic subjects*	Control subjects†	P
GBM Width	428 ± 77	342 ± 52	<0.001
Vv(Mes/glom)	0.22 ± 0.05	0.20 ± 0.03	<0.001
Vv(MM/glom)	0.10 ± 0.03	0.08 ± 0.02	<0.001
Vv(MC/glom)	0.09 ± 0.02	0.09 ± 0.02	0.518
Sv(PGBM/glom)	0.14 ± 0.02	0.13 ± 0.02	<0.001
GlomVol	1.50 ± 0.53	1.71 ± 0.85	0.195
TFS	0.21 ± 0.07	0.23 ± 0.13	0.400
Vv(Int/cortex)	0.09 ± 0.03	0.13 ± 0.04	<0.001

Data are means ± SD. *n = 243 except for Vv(Int/cortex) (n = 204) and GlomVol and TFS (n = 196); †n = 87 except for Vv(Int/cortex) (n = 26) and GlomVol and TFS (n = 30).

TABLE 3
Percent of abnormal morphometric parameters in relation to duration of diabetes

Variable	Abnormal value*	Duration of type 1 diabetes			All durations
		2–8 years	8–14 years	14–20 years	
n		144	74	25	243
GBM	>445	23	50	68	36
Vv(Mes/glom)	>0.25	10	16	48	16
Vv(MM/glom)	>0.11	15	31	68	25
Vv(MC/glom)	>0.13	3	3	12	4
Sv(PGBM/glom)	>0.16	28	19	4	23
Sv(PGBM/glom)	<0.10†	1	5	20	5

Duration data are %. *>95th percentile; †<5th percentile of normal control subjects.

TABLE 5
Comparison of patients having an IAH of 1 and those with IAH >1

Variable	IAH = 1*	IAH >1†	P
Duration of diabetes	7.4 ± 4.0	10.0 ± 4.6	0.0003
GBM width	416 ± 70	475 ± 85	0.0001
Vv(Mes/glom)	0.21 ± 0.04	0.24 ± 0.06	0.0048
Vv(MM/glom)	0.10 ± 0.03	0.11 ± 0.04	0.0075
Vv(MC/glom)	0.09 ± 0.02	0.09 ± 0.021	0.1263
Sv(PGBM/glom)	0.144 ± 0.022	0.131 ± 0.023	0.0010
GlomVol	1.49 ± 0.50	1.60 ± 0.66	0.2213
TFS	0.21 ± 0.07	0.21 ± 0.08	0.5558
Vv(Int/cortex)	0.09 ± 0.03	0.10 ± 0.03	0.0840

Data are means ± SD. *$n = 178$ except for GlomVol, TFS ($n = 150$), and Vv(Int/cortex) ($n = 156$); †$n = 44$ except for GlomVol, TFS, and Vv(Int/cortex) ($n = 41$).

lower and GBM width was higher in Paris. Vv(MM/glom) was not significantly different among the centers. Sv(PGBM) and TFS (Table 7) were lower in Paris and Minneapolis than Montreal. However, there were no differences in the ratio of TFS to GFR among the three centers. The Paris center also had lower IAH than the North American centers (Paris versus Montreal crude odds ratio 0.22; 95% CI 0.05–0.96).

Relationship between morphometric findings and renal function. Hyperfiltration was found in 65% of the diabetic subjects, and this percentage was constant regardless of diabetes duration. Subjects with elevated baseline GFR (>130 ml · min^{-1} · 1.73 m^{-2}) had higher values for SvPGBM (0.143 vs. 0.137, $P = 0.025$), systolic BP (SBP; 115.2 vs. 110.5 mmHg, $P < 0.001$), and RPF (718 vs. 555 · min^{-1} · 1.73 m^{-2}, $P < 0.001$) than those with normal GFR. These differences remained after adjusting for age by multiple linear regression. No significant differences in other morphometric measures were related to GFR, RPF, or FF. GFR uncorrected for BSA correlated with TFS ($r = 0.31$, $P < 0.0001$).

Mean AER during the first year of the study correlated positively with Vv(Mes/glom) ($r = 0.14$, $P = 0.034$) and Vv(MM/glom) ($r = 0.16$, $P < 0.05$), but not with GBM width or Vv(Int/cortex). The eight subjects with microalbuminuria had greater GBM width, Vv(Mes/glom), and Vv(MM/glom) (Table 8).

DISCUSSION

This study represents the first large-scale attempt to describe the early natural history of DN in young type 1 diabetic patients. The study is longitudinal in design and examines the demographic and clinical factors that influence the highly variable rate of development of DN lesions. The concept of variability in the rate of development and progression of diabetic nephropathology is central to understanding DN risk and pathogenesis. Previous studies of identical twins discordant for type 1 diabetes showed that all nondiabetic twins have glomerular structural parameters in the normal range (20). Some of the diabetic twins, despite long-standing type 1 diabetes, also had glomerular structural parameters in the normal range, whereas others had abnormal measurements. However, regardless of whether their glomerular structural values were normal or abnormal, in every instance, the diabetic twin's values for GBM or TBM width and Vv(Mes/glom) were greater than in their nondiabetic twin (20). Those data, comparing genetically identical individuals, suggest that all long-standing type 1 diabetic patients have changes in renal structure, but in some, the rate of change is so slow that their values do not rise above the normal range despite many years of diabetes. Thus, all diabetic patients appear to have the same direction of glomerular changes and vary primarily in the rate at which these changes develop.

This study, longitudinal in design, will estimate the rate

TABLE 4
Baseline predictors of renal structural variables

Variable	GBM Width		Vv(Mes/glom)		Vv(MM/glom)		Vv(MC/glom)		Vv(Int/cortex)	
	Adjusted effect	P	Adjusted effect	P	Adjusted effect	P	Adjusted effect	P	Adjusted effect	P
Duration of diabetes (per year)	7.2	<0.001	0.0035	<0.001	0.0027	<0.001	0.0004	0.205	0.002	0.0002
Female sex	−2.5	0.776	0.0099	0.062	0.0091	0.011	−0.0001	0.966	−0.003	0.0459
HbA$_{1c}$ (per mean % in first year)	6.0	0.076	−0.0002	0.937	+0.0021	0.118	−0.0021	0.047	0.003	0.0177
DBP (per mean mmHg in first year)	2.3	<0.001	0.0011	0.008	0.0008	0.003	0.0002	0.348	0.001	0.0022
Center										
Minneapolis (vs. Montreal)	−8.1	0.401	0.0006	0.919	−0.0038	0.339	0.0038	0.211	+0.002	0.6404
Paris (vs. Montreal)	29.8	0.018	−0.0242	0.002	+0.0010	0.844	−0.0242	<0.001	−0.006	0.264

TABLE 6
Comparison of patients with <5% GS and those with >5% GS

Variable	<5%*	>5%†	P
Duration of diabetes	7.7 ± 4.1	10.8 ± 5.4	0.0047
GBM width	425 ± 77	475 ± 71	0.0104
Vv(Mes/glom)	0.22 ± 0.04	0.25 ± 0.07	0.1124
Vv(MM/glom)	0.10 ± 0.03	0.12 ± 0.05	0.1694
Vv(MC/glom)	0.09 ± 0.02	0.10 ± 0.03	0.0406
Sv(PGBM/glom)	0.14 ± 0.02	0.13 ± 0.03	0.0084
GlomVol	1.48 ± 0.53	1.72 ± 0.45	0.0950
TFS	0.21 ± 0.08	0.21 ± 0.05	0.8406
Vv(Int/cortex)	0.09 ± 0.03	0.11 ± 0.03	0.0186

Data are means ± SD. *n = 207 except for GlomVol, TFS (n = 174), and Vv(Int/cortex) (n = 179); †n = 17 except for GlomVol, TFS, and Vv(Int/cortex) (n = 16).

TABLE 8
Comparison of baseline morphometric findings in diabetic patients with microalbuminuria and normoalbuminuria

	Micro-albuminuria	Normo-albuminuria	P
n	8	213	
GBM width	510 ± 93	425 ± 78	0.0029
Vv(Mes/glom)	0.28 ± 0.07	0.22 ± 0.04	0.049
Vv(MM/glom)	0.14 ± 0.05	0.10 ± 0.03	0.049
Vv(MC/glom)	0.10 ± 0.03	0.09 ± 0.02	0.395
Sv(PGBM/glom)	0.13 ± 0.03	0.14 ± 0.02	0.138
GlomVol*	1.69 ± 0.99	1.50 ± 0.51	0.663
TFS*	0.20 ± 0.13	0.21 ± 0.07	0.872
Vv(Int/cortex)†	0.10 ± 0.02	0.09 ± 0.03	0.589

Data are means ± SD. *n = 6 for microalbuminuria and 175 for normoalbuminuria; †n = 7 for microalbuminuria and 175 for normoalbuminuria.

of development of diabetic renal lesions based on two biopsies 5 years apart. The cross-sectional data presented here assumes that structure was similar in all patients at onset of diabetes. However, it is known that age influences renal structure, and this is particularly relevant to the present report because about half of the patients were between 10 and 18 years of age (21). Moreover, the range of normal values at any age is wide (21) and, as pointed out earlier, patients may be developing DN lesions and yet remain within the normal range (20). It should also be pointed out that multiple statistical comparisons were performed without Bonferroni or other corrections, and thus some of the statistical associations may have arisen by chance, particularly sex or center differences, which were not hypothesized a priori. However, the possibility of type 1 error is far less likely where the associations found were expected. Despite the limitations of these assumptions, several interesting observations emerged from these cross-sectional data.

All but 8 of the 221 patients with sufficient data for classification were normoalbuminuric. None of the comparisons between diabetic patients and control subjects for any of the structural variables were altered when only the normoalbuminuric patients were considered. Although 87 normal control subjects were studied, the number was insufficient to define age-specific cutoffs for normal values. Thus, the frequency of increased GBM width in the type 1 diabetic patients <18 years of age may have been slightly underestimated. GBM width and Vv(Mes/glom) were increased in the diabetic patients, and the proportion above the normal range increased with increasing diabetes duration. The major reason for the increase in Vv(Mes/glom)

was increased Vv(MM/glom), with no contribution from the mesangial cellular component. These findings are consistent with our previous reports indicating that, as a group, long-standing type 1 diabetic normoalbuminuric patients have significant diabetic glomerulopathy lesions (5,9,22). They are also consistent with findings of Ellis et al. (23) that normoalbuminuric subjects, including prepubertal children with average duration of ~8 years, often have GBM thickening and mesangial expansion. Berg et al. (24) found greater GBM width and Vv(MM/glom) in 36 normoalbuminuric adolescents compared with control subjects, but Vv(Mes/glom) was not increased.

Using pooled data from several studies performed in her laboratories, Østerby (25) also described increased GBM width but no increase in Vv(Mes/glom) in nine normoalbuminuric type 1 diabetic subjects with a mean diabetes duration of 12 years. Although some of these differences may be due to diabetes duration, the duration in the normoalbuminuric subjects in this and other studies (23) was even shorter than in the studies cited above, where mesangial expansion was not found. Differing study outcomes could be related to differences in glycemic control, center differences such as those encountered in the present study, or the much larger number of subjects in this report. Nonetheless, it is clear from the present study and previously published work that significant diabetic lesions are often present in normoalbuminuric subjects within a few years after onset of type 1 diabetes (5,9,22,23,25,26). Although on average, as demonstrated here and previously, microalbuminuric type 1 diabetic

TABLE 7
Comparison of baseline biopsy parameters by study sites

Variable	Minneapolis	Montreal	Paris	P
n	85	117	41	
GBM width	415 ± 86	430 ± 67	449 ± 81	0.0565
Vv(Mes/glom)	0.22 ± 0.04	0.22 ± 0.05	0.19 ± 0.05	0.0008
Vv(MM/glom)	0.10 ± 0.03	0.10 ± 0.03	0.10 ± 0.03	0.5229
Vv(MC/glom)	0.10 ± 0.02	0.09 ± 0.02	0.07 ± 0.02	<0.0001
Sv(PGBM/glom)	0.14 ± 0.02	0.15 ± 0.02	0.14 ± 0.02	0.0082
GlomVol*	1.48 ± 0.93	1.54 ± 1.13	1.31 ± 0.84	0.2038
TFS*	0.20 ± 0.23	0.23 ± 0.19	0.19 ± 0.11	0.0182
Vv(Int/cortex)†	0.10 ± 0.03	0.09 ± 0.03	0.09 ± 0.02	0.3415

Data are means ± SD. *n = Minneapolis (68), Montreal (109), and Paris (19); †n = Minneapolis (68), Montreal (109), and Paris (27).

subjects have worse glomerular lesions than normoalbuminuric subjects, there is considerable overlap in structure between these two groups. The increase in GBM width and the fact that most of the increase in Vv(Mes/glom) is due to increased Vv(MM/glom) (6,25–27) are consistent with the central role of accumulation in the pathogenesis of DN.

Even after adjustment for BSA and age, GlomVol was not significantly increased in the diabetic subjects. To our knowledge, GlomVol has not been previously compared between largely normoalbuminuric type 1 diabetic and control subjects. Rudberg et al. (27) reported a trend toward glomerular enlargement in microalbuminuric type 1 diabetic adolescents, whereas Østerby et al. (28) found increased GlomVol in microalbuminuric adults. Fioretto et al. (29) reported a trend toward increasing GlomVol over 5 years in 11 adults with long-standing type 1 diabetes with increasing AER and Vv(Mes/glom), and Ellis et al. (23) found a significant relationship between AER and GlomVol in children with ~8 years of type 1 diabetes duration. Taken together, these data are consistent with the hypothesis that little or no glomerular enlargement occurs early in type 1 diabetes and that glomerular enlargement may largely be a consequence of worsening glomerular lesions.

The finding that Vv(Int/cortex) was reduced by about one-third compared with control subjects in these largely normoalbuminuric diabetic subjects is both new and surprising. We have previously reported that Vv(Int/cortex) was increased in normoalbuminuric subjects after ~19 years of type 1 diabetes and that Vv(Int/cortex) increased further, with progression to microalbuminuric and proteinuria, especially if GFR was reduced (11). Thus, increasing Vv(Int/cortex) in type 1 diabetes may be a function of diabetes duration and may be associated with worsening glomerular lesions. We have recently shown that the initial increases in Vv(Int/cortex) in type 1 diabetic patients is due to expansion of the cellular compartment of the interstitium, whereas the increase in fibrillar interstitial collagen is a late manifestation of this disorder, occurring in patients whose GFR is reduced (30). It will be important to study interstitial fine structure in the patients presented here in order to better understand the earliest diabetic changes occurring in this important renal compartment. A possible explanation for this reduction in Vv(Int/cortex) is that the major compartment of the cortex, the tubules, could have expanded without a concomitant increase in the interstitial compartment. If true, and given that ~85% of kidney volume is occupied by tubules, kidney size would be expected to be increased in this cohort and, in fact, this was the case (13).

Because DN lesions develop slowly, it is not surprising that the prevalence of abnormal structural measurements increases with duration. Nearly half of our patients have mesangial expansion, and more than two-thirds have GBM thickening or increased Vv(MM/glom) after 14–20 years of type 1 diabetes. Increased filtration surface density [Sv(PGBM/glom)] was present in about one-third of patients early in diabetes, but this had decreased to <5% by 14–20 years. Reduced filtration surface density was rare in patients early in the disease but present in about one-fifth of patients with diabetes of longer duration. It has previously been shown that hyperfiltration is associated with

increased filtration surface (31) and that decreased filtration surface is likely a consequence of mesangial expansion (7).

Duration is an important predictor of DN lesions (4,9,27), and this was confirmed for GBM width, Vv(Mes/glom), Vv(MM/glom), Vv(Int/cortex), and IAH. Previous studies have not examined the effects of sex on these lesions. Our study showed female sex to be associated with a tendency for greater Vv(Mes/glom) and significantly greater Vv(MM/glom). This is likely an effect of the diabetes because these structural measures are equivalent in normal female and male individuals (21). These sex differences were independent of HbA_{1c} and emerged despite lower GFR, RPF, and SBP in the female subjects (13). The meaning of these findings is currently unclear, since there are no major sex difference for ESRD risk between male and female type 1 diabetic patients (1).

It was surprising that glycemia, as estimated by the average HbA_{1c} values in the first year of this study, was a relatively weak predictor of the baseline DN lesions. There was a trend toward a positive correlation with GBM width; a minor, albeit significant, direct relationship with Vv(Int/cortex); and a weak negative association with Vv(MC/glom). Similar findings of a correlation of HbA_{1c} with GBM width but not with Vv(Mes/glom) or Vv(MM/glom) in young type 1 diabetic patients have been previously described (23,24,27). Much stronger relationships between glycemia and rates of changes in glomerular structure have been reported in adults with microalbuminuria (27,32). Taken together, these results are consistent with the concept that a large subset of type 1 diabetic patients will escape DN complications despite poor glycemic control, whereas in the subjects susceptible to DN complications, glycemia is likely a major factor in the rate of development of lesions. Because the presence of microalbuminuria indicates a four- to fivefold increase in the risk of progression to overt DN (33), the study of microalbuminuric patients should select a more susceptible population and reveal stronger relationships to glycemia. Only a tiny fraction of the patients in this study were microalbuminuric at baseline; therefore, the influence of glycemia as a risk factor for DN will not be defined in our patients until the longitudinal studies are completed.

Considering that hypertension was an exclusion criterion for entry into this study, it is notable that DBP was a highly significant predictor of GBM width, Vv(Mes/glom), Vv(MM/glom), and Vv(Int/cortex). Ellis et al. (23) found similar relationships of DBP with interstitial but not with glomerular lesions. A recent study of 41 normoalbuminuric adolescents examined relationships of 24-h ambulatory BP and heart rate data and renal biopsy findings. Although univariate analyses indicated a relationship of nocturnal mean arterial pressure and GBM width, multiple regression analyses revealed relationships of HbA_{1c} and heart rate, but not BP, with GBM width and Vv(MM/glom) (34). This study also found greater GBM width in patients with nocturnal DBP above versus at or below the 90th percentile (34), but direct comparisons are not possible because hypertensive patients were excluded from the present study. Studies of microalbuminuric patients have reported relationships of glomerular structure and BP (5),

but these studies included hypertensive patients and patients with more advanced lesions, in whom hypertension could be the consequence of the DN lesions rather than the cause. Thus, this study suggests that variations of BP in the normal range in a largely normoalbuminuric cohort of young type 1 diabetic patients is predictive of diabetic glomerulopathy lesions. Longitudinal studies may clarify whether variability of BP within the normal range affects the genesis or occurs in response to DN lesions. Given the generally mild lesions seen in this study to date, the baseline data seem to be more consistent with the hypothesis that systemic BP may be causally linked to DN lesions and may be a risk factor early in the course of diabetes. This link may be direct, through effects of systemic BP on glomerular hemodynamics, or indirect, through genes linked to the propensity to develop essential hypertension (35,36). However, we found no relationship of FF with glomerular lesions (see below), suggesting that the relationship of BP to DN lesions may not be operating through increases in glomerular capillary pressure.

This study did not demonstrate relationships between GFR, RPF, or FF and DN lesions. This is in contrast to longitudinal studies suggesting that hyperfiltration is a risk factor for later development of microalbuminuria and for the later presence of DN lesions (27). This question will be reexamined in our follow-up studies.

Among the more interesting observations is the study site differences in renal structure. GBM width tended to be greater in Paris, whereas Vv(Mes/glom) was lower, primarily because Vv(MC/glom) was reduced. SBP and DBP were also lower in the Paris center, as were GFR and RPF (13). This combination of results is confusing. Studies in diabetic rats suggest that increased systemic BP is associated with increased GBM width (37), but the Paris patients had higher GBM width despite lower BP. The lower Vv(Mes/glom) in the Paris subjects is due to lower relative MC volume, and it is difficult to understand how lower BP, GFR, and RPF could produce this structural outcome. It is possible that study site differences are related to environmental and/or genetic differences. We found no differences in glycemic control or protein intake between the study sites (13), but other environmental factors could be operative. We have previously shown that not only the severity, but also the patterns of glomerular lesions, including the ratio of MM to MC fractional volumes, are concordant among type 1 diabetic sibling pairs who are known to be concordant for DN risk (38). Although the present study was not able to address these possibilities, it may help to explain why studies of early DN lesions have provided a variety of results.

In summary, this study has shown that *1*) DN lesions occurring in young normoalbuminuric type 1 diabetic subjects are significantly related to disease duration, but are less closely related to glycemia or renal hemodynamics than previously reported; *2*) systemic BP within the normal range may be a more important determinant of early DN lesions than previously appreciated; and *3*) important differences exist among study sites. Longitudinal studies currently underway in this cohort should help answer some of the questions raised by these cross-sectional analyses.

APPENDIX

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